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EVALUATION OF OPTICAL METHODS IN BIOMEDICAL RESEARCH

By

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The Prudential Insurance Company of America, Newark, N. J.

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Introduction

The original purpose of this paper was to survey recent developments in the field of optical instrumentation,† with the intention of clarifying them for workers in medicine and biology who possess a necessarily limited acquaintance with physics. However, a review of the material showed that, while there is a plethora of new devices and advances that are worthy of comment, these actually involve no really new principles or methods that have not been already described by greater authorities.

The following is a simplified exposition of the contemporary methods of optical design and evaluation. It will be seen that several concepts of value in the field of electronics have been successfully transposed to optics. For an understanding of this discussion, it is desirable that practical conclusions for the biomedical researcher be derived without retaining the mathematical complexity of modern, advanced optical computation. I have attempted this by drawing the analogies that exist between optical and electronic communication channels and by applying an elementary method of frequency analysis to their respective performances. Conversely, it is expected that, in conformity with the initial aim, some contemporary criteria for instrumental design, for performance evaluation, and for a more complete interpretation of the experimental data will become readily intelligible to the experimenter as a result of this analysis. For these purposes a brief interpretation of the most elementary principles of instrumental optics from the standpoint of the communication theory is offered first. I have previously outlined the historical development of optics as a science in Sumerian, Babylonian, Chinese, Hindustani, and

*This paper, illustrated with lantern slides, was delivered at the first meeting of the Instrumentation Section of The New York Academy of Sciences on January 20, 1959. The experimental work to which reference is made herein was supported entirely by the Physics and Physiology Branches of the Office of Naval Research, Department of the Navy, Washington, D. C., under contracts granted to the Paterson General Hospital, Paterson, N. J., and to the Columbia University College of Physicians and Surgeons, Department of Anesthesiology, for the construction of a continuous recording interferometer.

†The consolidation of various instrumental and conceptual fields into the new science of instrumentation originated in the pioneering work of the late M. F. Behar[1,2] and has been summarized by Condon.[3] Thus, there resulted a crystallization of certain general data, mental constructs, and principles common to all instruments and methods regardless of the nature of the quantities measured and the probes used.[4] Consequently, it now becomes possible to transpose the disciplines of the new science, as was done earlier with those of physics and chemistry, into the "scientific frontier territories"[5,6] or fields of investigations where "fundamental knowledge and basic understanding are still wanting" (Weiss[7]).

Greek antiquity to modern times and have demonstrated[8] its influence upon the philosophical thought of all ages.[8] Its major development was a true Greco-Latin miracle without parallel in other civilizations.‡

It is convenient to consider an observation as the ultimate result of some kind of interaction** between the object, the subject armed with his instruments, and some kind of discrete energy probe, all three working temporarily in perfect coordination. The subject's contribution is usually limited to F. W. Bessel's "personal equation." This interaction results in the transfer of a definite amount of information in the direction of the observer. Formally, to effect this transfer by optical means has no particular virtue except in exceptional cases, and it could be done as well by mechanical, acoustical, or electric (electronic) means. In all cases the same differential equation, with suitable units, governs each system and incorporates the same terms (inertial, resistive, restoring function, driving function, response function, and response rate). The equation is that of the transient and steady-state solution for a dampened harmonic oscillator. Demler[13] remarks that in most instances the choice is a matter of convenience, dictated by the applications considered. However, a strong point can be made in favor of optical methods in biomedical investigations. Indeed, the optical information transfer is effected by streams of photons or quanta of action, which are the finest probes available until something smaller than a quantum becomes known. Furthermore, such a discrete probe is endowed with periodic properties: it has a wave attached to it, and this offers definite advantages. Thus, optical research becomes a search for means of effecting the information transfer with maximum efficiency through instrumental channels designed in view of definite applications. Today optical engineering emerges as a complex science encompassing all devices involving the use of electromagnetic energy.

The remarkable necessity of providing an energy probe external to the biological open systems leads to a formal distinction between these systems and physical objects of investigation.[14] This requirement results in such complications as: (1) the impossibility of using primary effects for

‡For a brief outline of the historical development of the concepts of reflection and refraction of light the reader is referred to two of my previous publications.[9,10] The contributions of Thales, Epicurus, Leucippus, Democritus, and Plato are pursuing us today, for the Ionian philosophers, in their optical studies, had already attained the peaks of metaphysics, even before acquiring sufficient physics.

**The idea of generalized interaction originated simultaneously with the discovery of light refraction. Protagoras (5 B.C.) taught correctly that subject and object are always inextricably interwoven in scientific observations. This original idea apparently carried some tenacious parcels of truth, for it continually reoccurred each time science took a decisive turn. It is found in the works of Democritus, St. Augustine, Descartes, Kant, Leibnitz, Hamilton, and Mach. Schroedinger claims that "in final analysis, physical action is always an interaction."[11] A generalized interaction theory applicable to biological systems has been attempted by Stern.[12]

measurements, as done in physics; (2) the existence of speculative relationships between the usable most-reliable secondary effects and the phenomena sought; (3) very weak signals requiring high amplification (or magnification), with an inherently noisy channel drowning the useful information; (4) the incompatibility of both probes and transducers with an undisturbed steady state of the biomatter investigated; and others. These difficulties cloud the meaning of the results, which may remain behind the uncertainties created. The last circumstance, in particular, results always in chains of events or disturbances lasting long after the measuring paraphernalia are removed from the object, so that some of the results of the observation or measurement never reach the instrument and thereby the observer.†† In this field, one never can reach fully predicative conclusions.

Geometric Optics

The geometric treatment of optical image formation is a subject for continuous meditation, for very seldom do the final results exactly meet the expectations. The principal reason for this state of affairs is an incomplete understanding of the postulate of rectilinear propagation of light in homogeneous media. This postulate, at best, indicates the direction in which the image is found. It defines only the outside envelope of all the possible trajectories of radiant energy contributing to a given phenomenon. In this connection, de Broglie[17] remarks that, as long as the refractive index does not vary suddenly, the geometric treatment remains compatible with both the contemporary undulatory theory and Newton's strangely modern corpuscular conception of light. However, the geometric treatment can never fully account for the actual structure of the image.

From a practical standpoint, the limitations of geometric optics often lead to deliberate abuse of openings, apertures, diaphragms, and the like which more often than not, further deteriorate the image.

A second difficulty stems from an unwarranted assimilation of the real diopters of finite thickness with their geometric idealizations, which are devoid of physical body and of real thickness. Such simplified concepts result in a neglect of the real path lengths, so that unexpected artifacts appear in the images.

††It may be for this reason that one never makes anything but variation-of-tension measurements in biological systems, which are thus characterized by a perpetual "state of becoming." Regardless of the philosophical interpretation, the difficulty mentioned is probably the cause of the notorious lack of reproducibility of such measurements in irreversible systems in steady state.[14] The projection of these ideas into the study of open thermodynamic systems in the steady state[15] that do not obey Carnot's principle[16] (that is, most biological systems) was the theme of my Introductory Address to the Instrument Society of America Conference on Biomedical Instrumentation, held at New York, N. Y., on February 3, 1954. The subject was developed in a "Course on the Nature of Biological Information," given at the École Libre des Hautes Études, New York, N. Y., in December 1954.

The experimental demonstration of these points is done with the help of a punctual source, that is, a few pieces of cardboard pierced with different apertures and a viewing screen, in the order given. This elementary classroom demonstration illustrates such factors as the classic concepts of rays, the production of shadow and penumbra, and Ptolemy's law of reflection. However, it is seldom pointed out that it simultaneously demonstrates the impossibility of physically isolating a parallel ray of light by means of diaphragms alone. Due to diffraction, some light is always found in directions not defined by the apertures. Therefore, diaphragms incorporated in instruments cannot be used to determine the shape and direction of the rays. Their real functions are quite different. Conversely, it is always possible to form a bundle of parallel rays by means of a collimator – a punctual source at the focus of nondiaphragmed diopter. This last statement contains a good practical criterion of quality for the rapid evaluation of simple instruments.

The collimator is a convenient instrument with which to demonstrate what happens to the fraction of light that is not transmitted linearly. If the emerging light illuminates a small aperture, the latter transmits a maximum of light in the direction predicted by geometric optics, but there is also a considerable amount of radiant energy at considerable distances from the center. This last fraction is distributed unequally in the well-known diffraction fringes. The intensity distribution is given by the conventional Fresnel vectorial construction and M. A. Cornu's spiral diagram. With care, it is possible to observe light at 180° of the principal direction of propagation, as did Gouy originally. However, nothing happens if there is no diaphragm. Consequently, one concludes that diffraction is due to the interaction between the electromagnetic waves of light and the edges of the aperture; optical devices are always diffraction-limited communication channels. At this point another criterion of quality becomes apparent: the diffraction fringes of order greater than 1 should not be reflected by the walls of an instrument housing. This can be accomplished by giving attention to the design of limiting apertures and lens or mirror mounts and by the use of blackened coaxial tubes of various lengths and diameters instead of the usual internal flat shields found in many instruments.

The Babinet theorem. The simple experiment described can be used to derive a most startling theorem of physical optics, which unfortunately is seldom applied: the so-called Babinet theorem of complementary diaphragms.

The experiment is done with two collimators; the viewing screen is at the focus of the second. The diffraction image is essentially unchanged, whether the diaphragm is opaque or bears a small aperture. This is explained again by the contention that the phenomenon is due only to the edges of the aperture, and not its content (opaque or transparent). Thus,

in many cases when the aspect of the conjugate focus is unimportant or even disturbing, axial opaque operculi are often more advantageous than diaphragms. Examples of such applications will be reported.

The importance of diffraction in the design of specialized instruments was emphasized in a previous publication.[18a, 18b] For instance, it was shown that the ideal slit* with a Shuster factor (e) mm = 1. $f\ \lambda/4\ D$,† so desirable in spectrophotometry, can preferably be made 50 times larger in an interferometer, provided that (1) the lens diameter D is kept small, (2) all extreme rays have an incidence smaller than 10°, and (3) the focal length f is longer than 5 D.

A direct application of Babinet's theorem is found in the microscope interferometer previously described.[19] In this instrument it is possible to gain enormously in luminosity by opening the diffracting twin slits far more than is obviously reasonable for, in reality, only the external edges of these slits do not serve any useful purpose beyond cutting off the excess of diffused light, and this can be best achieved by other means.

Principle of de Fermat and the wave front surface. In solving design problems it is seldom sufficient to consider ideal elements devoid of volume or to limit the reasoning to the homofocal conical meridians of the reflecting or refracting surfaces. Even with stigmatic surfaces by reflection it is important in some cases to account for the short penetration of the radiations into the material of the reflector as well as into that of the receptor or the transducer.

One demonstrates that in simple cases with parallel light the algebraic sum of all the partial path lengths of incident plus reflected rays remains constant regardless of the incidence for a given surface, be it an ellipse, an hyperboloid, or a parabola. One shows also that the ideal stigmatic surface may be different from the actual physical surface of the diopter. The same conclusions apply to diopters acting by refraction. The optical path length from the object to its image is defined by the product of the geometric distance by the refractive index. Such considerations are at the foundation of the principle of Pierre de Fermat;‡ the interpretation of this principle, however, requires some care. The principle states only that the sum of all optical path lengths followed by the light rays through

*Such a slit produces a retardation between its two edges respectively and one edge of the conjugated collimator lens of only $\lambda/8$. A condensed table of spectral purity and slit luminosity versus e is contained in the publication listed.

†Lens diameter.

‡This "principle of stationary time" is usually expressed in the form: $\delta \int n ds = 0$, or: $\delta \int ds/d\lambda = 0$. For a short discussion of its historical role in correcting Renee Descartes' original law of refraction, the reader is referred to a previous publication.[8] It is worth noting that a very similar principle had been formulated in the Third Century B.C., by Hero of Alexandria who implicitly admitted a finite light velocity.

any succession of stigmatic surfaces is stationary; that is, two such adjacent rays differ in optical length only by an infinitesimal quantity, at least of second order. The principle does not state whether this length is a maximum, a minimum, or an inflexional quantity, but only that it is constant. In fact, it can have any one of these three characters according to the circumstances (or whether the actual refracting surface is tangent external, internal, or secant to the calculated stigmatic surface, respectively, in the order given above).

The implications of this principle pervade much of physical chemistry. Almost simultaneously with its formulation, Pierre Maupertuis demonstrated that, when a material point in action is analyzed, the sum of all the energies involved is always stationary, and the representative equation is $\delta \int \sqrt{(U + E)\, ds.}$ † The analogy with the stationary time equation is now evident. However, it required the genius of Sir William Hamilton to discover it, by postulating that $\sqrt{(U + E)} = n$. Thus, the refractive index n expresses the force function or field existing in matter, which results in slowing down the velocity of light and determines the trajectory of rays or streams of information, to use modern terminology. In this sense, n is a measure of interaction between matter and light, and its determination contributes to a knowledge of the structure of matter. The abstract concept of a field of forces remains only a convenient but arbitrary form of language to explain the properties of space and to predict the future behavior of the material particles, the local sources of the physicists, or points of particular chemical interest contained in space. Thus, field of forces, space properties, and the chemical properties of the elements are theoretical views of the mind that become real, like the shadow of a tree, only when some interaction takes place. One sees that the concept of generalized interaction is contained in that of field, as was perceived by Paul Langevin when he wrote in 1903: "It is always matter which contains the charges whose field divergence becomes different from Zero." In the absence of matter, indeed, $\sqrt{(U + E)} = 1$, and it is the nature of the sources that determines the properties of the field: if there is no matter, there are no charges, no sources, no force, and no field in space.

The application of the principle to optical research is simplified by the introduction of the Malus theorem, which states that the rays emerging from a stigmatic surface constitute a congruence of normals. In ordinary language, this means that all the rays contributing to the image fall perpendicularly upon a surface Σ, each point of which is equally distant optically from the conjugate object, such a distance being given by de Fermat's principle. From the practical standpoint, such a surface of constant optical phase is the wave-front surface. This surface for a per-

† U = potential energy, E = kinetic energy, and ds = distance.

fect collimator, for instance, is a plane. The existence ot a congruence of normals is due to the fact, now evident, that the direction of propagation of radiant energy is defined by a series of parameters, x, y, and z, which depend upon at least two independent variables (v, u, and others). If only one independent variable is considered, one has a family of curves. This is the case of geometric representation: if n is constant, all the rays of wave propagation considered in geometric optics form a totality of curves orthogonal to a family of wave surfaces. The theorem is useful in calculating the results expected from real systems and for establishing corrections for the various aberrations. In general, it is possible to derive formulae that describe the image pattern in terms of the deviations of the actual wave front surface S' from an ideal reference sphere S. This method encompasses at once all aberrations of the first order (transverse and longitudinal focal shift or errors of adjustment) and of the third-order (spherical aberration, astigmatism and caustics, coma, distortions, and field curvature). It is obviously unnecessary to include a complete derivation of the aberrations in this discussion. I shall try to show that intelligible and simple criteria of instrumental quality and performance can be formulated without going into the mathematical details of the aberrations, provided the criteria are chosen each time in view of specific applications.

The easiest approach to the problem is through a method of frequency analysis common to process engineering, optical engineering, and data processing, as well as to the unraveling of the information content of complete laboratory data. The only initial postulate made to simplify the reasoning is that any optical system can be compared, for the purpose of frequency analysis, to the black box of the electrical engineer. Here, the black box is limited by the position of the two omnipresent entrance and exit pupils defined by Nijboer.[20]

Frequency analysis of optical systems. The definition of object-image relationships in terms of frequencies is not new. It was attempted as soon as it was realized that the microscope image was in reality a diffraction image more readily explainable in terms of the wave theory of light than by means of geometric optics. The practical application of the concept of spacial frequency distribution in the object space, however, dates from about 1940. To illustrate it in simple terms, one considers an object exhibiting a sinusoidal intensity variation in a direction forming an angle θ with the x axis, this angle thus defining another direction x'. The object is "seen" along the z axis. The angle θ is introduced for purposes of generalization. Supposing that the intensity peaks are repeated every P mm., for instance, and considering the one-dimensional problem for the moment, the intensity distribution on the extended object plane O can be represented by a simple equation:

$$O\,(x', y') = c.e^{+i.w_o x'} \tag{1}$$

where one postulate: $w_o \doteq 2\pi/P$.

The spacing P appears as the reciprocal of the frequency: $1/\nu$ (the object contains ν peaks per millimeter spaced P mm. apart). Thus, the quantity w_o and its cartesian coordinates, w_x and w_y, have the dimensions of reciprocal lengths. This spacial frequency has for trigonometric coordinates, respectively:

$$x' = x \cos\,\theta + y \sin\,\theta \tag{2}$$

$$y' = x \sin\,\theta + y \cos\,\theta \tag{3}$$

Consequently, the intensity distribution in the object space can be rearranged according to the following equation:

$$O\,(x,y) = c.e^{+\,w_o\,(x\,\cos\,\theta\,+\,y\,\sin\,\theta)} \tag{4}$$

where

$$w_{o,x} = w_o \cos\,\theta, \quad \text{and} \quad w_{o,y} = w_o \sin\,\theta \tag{5}$$

Neglecting the effect of magnification, if present, a linear optical system illuminated with incoherent light is characterized by the formation of an image whose intensity distribution $i(x,y)$ is also given by the above equation. The complete mathematical development demonstrates that, while the image exhibits the same spatial frequency distribution, it is shifted in space by an amount ϕ. It also shows that the amplitudes are modulated by a factor $|r|$. This factor is related to the transfer function of the system or its frequency response characteristic r by a simple relation:

$$r = |r|\, e^{-i\phi} \tag{6}$$

The net effect of the factors ϕ and $|r|$ is a reduction of the contrasts in the image space as compared to the object, and it is a measure of this reduction.

The practical aspect of the method becomes apparent if one observes that the transfer function is the same for a periodic object and for a nonperiodic object scanned by a small aperture (either unidimensionally or bidimensionally). Evidently, very important applications correspond to the last case (for example, teletyping and telephotography, television, and several kinds of scanning microscopic methods). One particular case involves the continuous scanning of a field of fringes of interference and is discussed elsewhere.[18a, 18b]

The above general theory does not take into consideration the periodicity of light, at least in this elementary form. However, some kind of relationship must be found if conclusions of a practical value in instrument design are to be formulated. One important problem is the effect of the mechanical limitations of instruments, such as limited aperture, supporting frames, and the finite size of lenses, upon the resolution limit.

Spatial frequency at the first resolution limit. In the most general case, an object characterized by a spatial period P is scanned with a small aperture of diameter $2r$. One finds experimentally that the periodicity of the object disappears in its image for a finite ratio $2r/P$. The mathematical theory indicates that this ratio is numerically equal to that of the first root of the first-order Bessel function (when $J\,3.83 = 0$) over π. This value of 1.22 corresponds to a sine-wave resolution w_o (in rad./length) given by EQUATION 7.

$$w_o = 2\,\pi/P = 3.83/2r \tag{7}$$

from which one finds that

$$2r/P = 1.22 \tag{8}$$

Marechal[21] remarks that the quantity $2\pi/P$ is in reality the modulus of the wave-vector $\vec{K}$ defining the direction and the phase of the sinusoidal structure. One may note also that the quantity $3.83/2r$ corresponds to the Rayleigh resolution limit for two independent points, so that EQUATION 8 can be rearranged to give the corresponding angular separation a:

$$a = 3.83\ \lambda/\pi\ 2r = 1.22\ \lambda/2r \tag{8a}$$

These relations show that the scanning aperture must be at least 1.22 times larger than P if the periodicity of the object is to be detected in its image. Indeed, when scanning interference fringes localized at the focus of a decollimating lens, it is found that the periodicity of the energy distribution disappears when this relationship between scanning slit width and fringe spacing is approached.

One may consider next the role of the aperture alone. The reasoning is easier with a narrow slit whose two edges, of spacing A, form two independent diffraction patterns when illuminated with incoherent light. A lens L is placed behind the aperture. To simplify matters, let us suppose it perfect and infinitely thin. Thus, the edges are imaged in air of refractive index $n = 1$, at the focal distance f. Each image is a Fresnel diffraction spectrum. By virtue of Babinet's theorem, the pattern is identical with that produced by a unique wire of diameter A in the plan of the slit. In the case of two small pinholes, one would observe two Airy discs. As the width

of the slit is reduced further, the first dark line of each pattern eventually becomes contiguous. The position of the successive minima in each spectrum can be calculated by the Airy method (integration by a convergent series), or by means of Bessel functions.* It can also be determined graphically, as did Schwerd in the last century, by mechanically resolving a circle of radius A into a large number (several hundreds, if possible) of equal trapezes.[22] One finds that the patterns become contiguous when the product A. sine a is equal to a finite fraction of the wave length λ, so that

$$A/2 = B.\ \lambda/\sin a \tag{9}$$

the calculation giving for B: 0.61. When this is attained, the angular diameter of each first-order minimum, sine a, is equal to the spacing of the two contiguous patterns, and the edges of the slit are fully resolved. Since the angle a is very small, its value can be equated to its sine value (a and λ being then expressed in mm.).** It must be observed that the example given is identical with the formation of the diffraction image by the first lens of a microscope immersion objective; onlv the ravs are traced in reverse direction (that is, a medium of refractive index n larger than unity would be interposed between the slit object and the lens). The conclusion remains valid, by virtue of Ibn-al-Haitham principle of the inverse return path of spherical wave disturbances (7 A.D.). Consequently, the quantities $2a$ (first minima spacing) and A are interchangeable, and the converted product, n. sin A, becomes the familiar Abbe numerical aperture of the system of focal length f limited by the entrance slit of half-width $A/2$. This transformed equation, $a = 1.22\ \lambda/n.\ \sin A$, gives the angular separation (a) of the points at the Rayleigh resolution limit. It is not the ultimate resolution of the system.

As A is further reduced, eventually the first-order minimum of one pattern will overlap with the central maximum of the other, and the periodicity of the image will no longer resemble that of the object. In practice, the double image disappears almost completely. This is achieved when

$$A = 0.61\ \lambda/n.\ \sin a \tag{9a}$$

*The fundamental assumption of geometric optics is really that the wave length be small compared to the dimensions of any other change in the media transversed by light. As the wave length tends toward zero, one arrives at the eikonal equation of geometric optics: (gradient $s)^2 = n^2$, which expresses that the surfaces of constant s value considered in de Fermat's principle define, again, the wave front.

**The expression is comparable to that used in astronomy to obtain the solid angle of view a, of a small object at the (infinite) distance z as a function of the telescope diameter O: $a = O/z^2$.

(that is, when the minimal spacing is equal to one-half the object pattern spacing). Somewhere between these extremes comes the effective slit width $x = 0.886\ \lambda\ .f/ds$, which is so important in spectroscopy. In this expression f is the focal length and ds is the distance between two points on opposed sides of the central diffraction fringe where the energy is 50 per cent of the central maximum.

It is now possible to find the first resolution limit P in the object-space of a system limited by a slit of spacing A. From EQUATION 8 it is found that $P = A/1.22$ when the periodicity vanishes. Replacing A by its value found in EQUATION 9a when the periodicity also vanishes, one finds that

$$P = 0.61\ \lambda/1.22\ n.\ \sin a = \lambda/2\ n.\ \sin a \qquad (10)$$

The spatial sine-wave resolution w_o of the system is again given by

$$\vec{K} = w_o = 2\pi/P = 2\pi\ .2\,n.\ \sin a/\lambda \qquad (11)$$

and the linear resolution R_s (in lines per millimeter) is, as before

$$R_s = 1/P = w_o/2\pi \qquad (12)$$

the quantity $1/\lambda$ being the wave number of the spectroscopists.

One may now remark that the quantity $2n.\ \sin a$ is equal to the dimensionless term $1/F$, F being the familiar f-number of the lens, so that $1/F = A/f$. Two important practical conclusions follow immediately: first, the ultimate resolution of the system forming an image at a finite distance with incoherent light is fixed solely by the wave length and the f-number. Second, it is possible to characterize any linear incoherent optical communication channel behaving as a low-pass frequency filter by its cut-off frequency R_s in lines per millimeter given by

$$R_s = 2n.\ \sin a/\lambda = 1/\lambda\ f \qquad (13)$$

From the above discussion, it is now evident that the sine-wave resolution of such a system is better than Rayleigh's resolution (compare EQUATION 8a).

Referring to EQUATION 9a, one concludes in addition that, if the limiting angle of rays that can enter the apparatus is approximately the same as the angle of diffraction of a light wave from the periodic object, then the spacing in the object space is just about the same as that of two points that can be completely resolved in the image.

It must be kept in mind that the term resolution does not imply a knowledge of the structure of the object, but merely an inference of its discontinuities with a quantitative evaluation of the sinusoidal intensity peaks

corresponding to these discontinuities. A more complete restitution of the object is obtained when the instrument admits rays corresponding to greater diffraction angles (larger $N.A.$), as is well known. Then, the device performs really a harmonic reconstruction of the object space.[23] Detailed developments will be found in the works concerned in particular with the generalized theory of microscope images and in those dealing with phase microscopy.[24]

The foregoing considerations hold when the adjacent elements in the object space are independently illuminated (that is, when completely incoherent light is found in the entire system). It is easy to show that the resolution R_s falls off sharply when the above condition is not realized or when coherent light is deliberately used. In this case the resolution limit is hardly larger than $\lambda\ 0.21/N.A.$, but it may be raised to a little above $0.50\ \lambda/N.A.$ if oblique illumination is employed.

The cases of spurious resolution fall outside the scope of this discussion. They are, however, fully explained by the same frequency-analysis procedure. The simplified exposition of this method points to relatively easy means of rating optical instruments with fairly accurate results in most instances.

Quality criteria. It is perhaps evident from the foregoing that several of the parameters entering into the evaluation of optical images are mutually exclusive, like canonically related variables. Thus, the quality criteria required of a given instrument depend upon a proper weighing of these parameters, and this can be done only by considering each particular application. The method of frequency analysis permits the separation of several such criteria of a statistical nature, which are easily accessible experimentally.*

The relative structural content T is a qualitative requirement with emphasis on sharpness of detail. One does not require an exact line-up of detail in the object and its image. The degree of achievement is measured by the mean square fluctuation of intensities in the image i, over that in the object O. Thus,

$$T = \overline{i^2\ (x,y)} \Big/ \overline{O^2\ (x,y)} \tag{14}$$

The fidelity, $FF = (1-D)$, is a requirement that the peaks and troughs in the image correspond exactly with those in the object. This quantity is measured by the sum of all the aberrations. This sum is obtained experimentally by determining the ratio of the intensity error distribution in the

*The corresponding tolerances are evaluated in terms of the Strehl definition, which involves a statistical comparison of the actual image with that produced by, or calculated for, a perfect instrument.

object-image spaces produced in the real instrument over the mean square distribution of a perfect instrument.†

Thus,

$$D = \overline{[O\,(x,y) - i\,(x,y)]^2} \,/\, \overline{O^2\,(x,y)} \tag{15}$$

The correlation quality factor Q of Linfoot[25,26] is given by using the same symbols:

$$Q = \overline{O\,(x,y)\,.i\,(s,y)} \,/\, \overline{O^2\,(x,y)} \tag{16}$$

The sharpness factor of O'Neill, S, is also expressed in terms of Strehl definition, so that the comparison with an ideal instrument S_o gives

$$s = S/S_o = \overline{[\text{gradient } i\,(x,y)]^2} \,/\, \overline{\text{gradient } i_o\,(x,y)]^2} \tag{17}$$

Referring to page 000, it is clear that S is related to the frequency response of the system, and that it may be manipulated to suit the requirements of each particular application.

In using these factors, one notes that three of them are related to one another as follows:

$$Q = (T + FF)/2$$

The fourth, independent criterion S, is interesting from the standpoint of information theory. By properly manipulating it, it is possible to preserve or emphasize the edges of details in the image. Overemphasis, however, can create confusion, and this is precisely one of the main drawbacks of phase-contrast microscopy, which penalizes excessively the low frequencies in the object space, thus requiring a re-education of the observer. Indeed, the importance of edge details is supported by strong psychological evidence, and the theory indicates that edges are the regions of maximum information content.

From an experimental standpoint one can derive the three following rules: (1) at low frequency the aberrations affect the filtration by a factor of second order relatively to frequency; (2) near the cut-off frequency the

†The alternate procedure involves the progressive correction of an imperfect instrument on the basis of successive sets of experimental checks of its performance until all E_o are eliminated. The calculations involve only arithmetical manipulations of sine and cosine ratios, but are quite lengthy. Only recently automation methods of punched-card encoding and processing on small computers (such as an IBM 626) have been applied to this problem. With their help, the calculation, design, and verification of optical devices are brought to a degree of perfection approaching the limit imposed by the nature of the radiation utilized.

effect is still smaller; and (3) the effect of aberrations is, on the contrary, very rapid at all intermediate frequencies.*

There are, of course, other methods of quality rating. Some involve a direct application of information theory, and it is not intended to exhaust this subject of discussion. It is expected that the foregoing makes it clear that the choice of a suitable method depends, in the final analysis, upon the applications considered, provided the requirements of such applications are quantitatively expressed in the terms of the method of frequency analysis. The usefulness of this concept is further illustrated by the sampling theorem.

The sampling theorem. The analogies between electrical and optical systems began to be considered seriously about 1950, following the work of Cheatham and Kohlenberg[27] and others.† Such analogies raise a number of questions relative to random functions in linear systems, the filtering of transients from noise, and even the definition of noise. Their solution is important in the case of optical systems performing scanning operations such as pinhole scanning, electronic imagery, photography of moving objects, photographic restitution, television, and X-ray diffraction by photography.

For a simple treatment, it is convenient again to describe the object and its image as successions of repetitive periodic functions (cycles), or "lines" of finite spacing P. Thus, the object function is defined by a set of frequencies such as R_s lines/mm. $= 1/P$, as above. The sampling theorem states that such a function $f(x)$ containing no frequencies higher than the upper band-pass R can be completely determined by giving its ordinates at a series of N_p points spaced $1/2R$ mm. apart, the series extending throughout the entire space domain. The mathematical demonstration is quite involved and is not relevant here. In practice, if the object function $f(x)$ exists over a length L, the total number of points required is $2r + 1 = 2RL$, the quantity on the right side defining the "degrees of freedom" of the system. From this point on, the fine ramifications of the sampling theorem are a fascinating subject, but can be treated only with the help of higher mathematics. One corollary, however, can now be readily understood by the experimenter. It concerns the relationships between optical image resolution and the finite size of any practical instrument.

*A simple graphical evaluation of the optical filter transmission factor Tr consists in drawing 2 circles whose centers are λ/P apart and whose radii are both equal to that of Airy disks at the resolution limit, or the as of above equations. Tr is measured by the overlapping areas of the 2 circles. Obviously, when $\lambda/P = 2a$, $Tr = 0$.

†See page 174, for justification of this position. The various mathematical methods available for deriving wave fronts of disturbance propagation in elastic media and in vacuum were recently summarized by Jardetzky.[28]

The resolution is expressed by EQUATION 13. This can be transformed into the following:

$$F = (1/R_s) / \lambda = P/\lambda \tag{18}$$

or the ratio of the ultimate detail size over the wave length. The quantity R_s thus appears as the ultimate limit of resolution in lines per millimeter, and the corresponding sampling interval becomes

$$1/2\,R_s = \lambda\,.F/2 = \lambda / (n.\ \sin a) \tag{19}$$

while the ultimate degrees of freedom remain unchanged, being defined by the diameter of the field of view L, which is limited by the physical size of the instrument. These relations hold well when observing either periodic objects or isolated details illuminated with incoherent radiations. The analysis is a little more complicated if one attempts to extend these ideas to include random distribution of details in the object. For the purpose of this discussion, however, it is sufficient to observe that the sampling theorem is based entirely on the low-pass cut-off of the optical system, for which maximum response occurs when $w = 2\pi/P = O$. In the case of random distribution there are an infinite number of functions that agree at the sampling points. Consequently it is more reasonable to discuss the performance in terms of sets of distributions all having the same statistical character. However, in so doing, one loses the phase information. Nevertheless, the ultimate result of the analysis is that, despite this loss, the input-to-output relations in terms of spatial frequencies remain unchanged, whether one deals with isolated, periodic, or randomly distributed details. This simple conclusion opens the way to the introduction of methods of noise filtration in optics of a rationalism parallel to that of the electronic methods.

In the case of a periodic object, a suitable sampling frequency based on that of the object, if known, results in filtering out all but a small percentage of the random optical noise, whatever its origin. Furthermore, the same considerations indicate that the filtering process must be initiated in the domain where the spatial frequencies are undistorted. If the object separation is known, the input pulse shape may be preserved exactly. However, in the most general case, only the pulse peaks can be determined exactly, while the shape is indefinite unless harmonic analysis permits its subsequent reconstruction.

In the case of isolated transients it is usually possible to determine only the peaks in the presence of noise. The maximum efficiency of the process is attained when the object is scanned with a pulse of the same shape if the latter is known. This result is almost intuitive.

In the case of a random-signal frequency spectrum there is extensive overlapping with the uncorrelated noise spectrum. The problem thus is of a statistical nature, and one can only attempt to perfect the performance. This is achieved by minimizing the average statistical error E_n to an arbitrary level, which must be stated. A convenient level to choose is the least mean square error criterion. Since the optical noise includes the effect of all types of aberrations, the method is akin to that of Marechal.[21 a,b] In this method one considers the wave front as a whole, in preference to analyzing its constituents separately, and one demands that the mean square error E_o around a reference sphere be a minimum.* The reference sphere is such that the E_o is 0.

Evidently, one could discuss the sampling theorem in the more general case of a totally unknown object that the observer tries to understand from the aspect of its image. The sampling frequency, that is, the number N of coefficients $A(m,n)$ of the Fourier transform, in the image domain is still determined by the physical size of the instrument. Linfoot remarks[25] that the number N' of sampling points available for a reconstruction of the unknown object is necessarily very much smaller in proportion to the magnification other than N in the object space, and only N' is a measure of the quantity of information received by the observer. Distance, in astronomical observations, plays a role similar to instrumental limitations. For instance, a distant galaxy with a diameter of 40,000 light-years contains $N = 10^{55}$ sampling points. If it is seen as one single Airy spot, its image contains only $N' = 1$ sampling point. Thus, the obstacles raised by unknown Nature between man and his objects of curiosity can now be also evaluated to some extent.[29-31]

Definition of optical noise. The practical utilization of the sampling theorem requires a knowledge of the physical nature of the noise. The definition is difficult. The noise includes a number of unrelated factors, some of which depend on the use to which a given instrument is put, such as diffused light, glare, flares, haze, polarization of various kinds, atmospheric turbulence, photographic grain, photodetectors, shot and thermal noises, some characteristics of human vision, and its own aberrations.† Many of these factors, if fully accounted for, would introduce marked nonlinearity in the system. From the standpoint of the experimenter it is convenient to group under the name of noise the combined effect of all the aberrations of the system alone, including the spherical and those due to chromatism, astigmatism, "barrel" aberration, and coma.

*There exists a simple relation between this mean square error and the Strehl definition mentioned above.

†For a short, comprehensive treatment of the visual aberrations and their role in instrumental observations, see Lapicque.[32]

One obstacle to the complete assimilation of an optical instrument to an electronic channel is that the optical noise suffers from several necessary restrictions in incoherent illumination. Even if the instrument possesses axial symmetry, the input and output functions always represent positive intensities in the entire domain. Furthermore, the signal and noise levels are not additive as in electronics but are, rather, mutually exclusive within the filter when it is physically possible to build such a device.

Incomplete as they are, the above considerations on the nature of optical noise serve to set the physical dimensions of instrumental components so that the signals sought, resulting from the object-subject-probe interaction, can be distinguished or at least recovered from the noise originating in the subject-instrument moiety of the system. Beyond such lower dimension limits, the scientific object of observation may vanish without the observer being aware of it,* for the instrument continues to transmit some information.

Optical filtration, distortions, and communication theory. The concept of optical instruments as linear communication channels leads to the investigation of the means of effecting this transfer of information with optimum efficiency. Thus, the general theory of information, together with the communication theory, should be applicable in this field. Such studies are not easy except in a few simplified circumstances, perhaps on account of the abstract character of the theories mentioned. The formal theory of information, which was originated by Frechet† and developed by Wiener,[60] by Shannon and Weaver,[33] by Quastler,[35] by Brillouin,[36] and others, can be used in the treatment of problems involving signals of a periodic nature, provided some restriction is placed on the term information. Originally, the "information content" H was a mathematical quantity defining the constraints of a system and related to the entropy S, to the configuration state, and to the Boltzman constant in a now well-known manner. While it is very desirable to arrive at a knowledge of the entire information content of the objects of scientific investigation, such a task presents insuperable difficulties, not the least of which is the evaluation of the information-carrying performance of the instruments used in making

*In addition to these difficulties, there exists a regrettable confusion of nomenclature, For instance, the term "white noise" has widely different connotations in electronics and in optics, depending on whether one considers spatial or temporal frequencies. This point has been discussed by Shannon and Weaver.[33]

†The concept of "distance function," which is akin to that of spatial frequency, was introduced by Frechet in 1906, a considerable time before the communication theory was in the embryonic stage. For details, see Zadeh.[34]

the observations. One may even question whether this total acquisition is desirable at present.*

The last factor may be termed the communicable information content of the operating system or, in brief, the capacity of the communication system. Thus this factor, like information and entropy, has a statistical character, and the study of optical communication systems takes on a unique character for, to paraphrase Sir J. J. Thomson[38] (1925), a solution of the problem of the transfer of energy through space at the same time brings a solution of the problem of the nature of light.

One of the simpler optical instruments has an aperture of area S traversed by a flux of radiations of wave length λ, carrying a quantity of energy E (ergs/sec./sq. cm.). The theory indicates that the maximum amount of information Q that can be transferred from a single source (or object detail) through the aperture is

$$Q = k.\ 4\,S/\lambda^2 \qquad (20)$$

k Boltzman constant ($1.38 - 10^{-16}$ erg/degree). Q is proportional to the aperture area S divided by one quarter of the square of the wave length and is finite for each wave, but is usually known only as a relative quantity or a ratio. Expressing S in terms of the aperture radius and comparing with EQUATIONS 8*a* and 9*a*, it is clear that the capacity Q is quantitatively related to the f number of the instrument and its numerical aperture NA and, therefore, to the resolution limit and to the sampling frequency (both in lines per millimeter). This quantity is the sum of all the kinds of information contained in the output signal. It contains the useful information fed to the input and the channel noise. Thus, it is natural to extend the theory to optical channels, despite very great difficulties, as pointed out by Cheatham.[38]

A survey of the contribution of information theory to optics requires developments beyond the scope of this presentation. However, the theory predicts a number of means and ways of filtering the noise, although the actual physical realization encounters difficulties that cannot be entirely solved by the methods of electronic engineering. Furthermore, it is worth noting that such predictions can be made more readily by inference from the theory than on the basis of its affirmative postulates.

One striking aspect of the theory is that no limit is set upon the number of radiation beams that can be efficiently transmitted by the aperture in the example considered. Thus, apparently, an unlimited number of inde-

*Schroedinger[16] already has remarked that the mere attempt to secure the minimum of meaningful observations to describe a single living object would so tamper with its performance that it would no longer be what was sought, hence predictability, in a causalistic sense, must escape us in biology.

pendent radiations of different wave lengths can be transmitted, each one carrying its own message (the situation may be different with astronomical observations, according to Duffieux[29-31]). The only problem is that of discriminating the various radiations at the output, both in amplitude and phase, if possible. This is the problem of fine resolution spectrophotometry. The simple considerations advanced above point to a possible way of improving ordinary, broad-band colorimetric measurements to the point of giving these routine measurements a high degree of precision, an absolute significance, and a satisfactory reproducibility, irrespective of instrumental divagations and deterioration of the apparatus from one laboratory to the next. It is always desirable to characterize instrumentally obtained data in terms that translate the physical interactions involved in the measurements, including a description of the biological or chemical systems studied.

At present, colorimetric performance is routinely evaluated in terms of the chemical reactions only, and interlaboratory discrepancies are adjusted by modifying the methods rather than by introducing corrective terms in the response of the instrument. An evaluation of the "residual cut-off energy"[39] is not sufficient for this purpose. While this quantity incorporates the molecular spectral absorbance of the substances being analyzed, it does not consider the behavior of the instrument under the actual conditions of each analysis. A more useful corrective term might be the relative absorption band intensity, I_r, defined by the empirical relation

$$I_r, \lambda = 100/C_x, \lambda \tag{21}$$

where C_x is a concentration of substance analyzed (in grams, per cent, moles, for example) capable of producing, at each band width of central wave length λ, an instrumental response representing an arbitrary level above the background (sum of chemical blank value plus total noise). A similar factor is employed in flame photometry to define the minimum line intensity of the elements attainable with each apparatus. For this latter application, where the background is always very high, x is usually limited to 0.5 per cent. In colorimetry x is preferably a small integer in the background. The next logical step is the evaluation of a "functional residual cut-off energy," which thus fully characterizes an instrumental analytical scheme. This problem is presently under investigation in the writer's laboratory with a view to developing general methods of biochemical analysis with complete automation of the equipment.[40-42]

The analogies between optical and electrical thermal noises are more than superficial. According to Smith,[43] the noise voltage V_n^2 generated within a linear electronic amplifier can be expressed by an equivalent fictitious thermal noise that would be produced in an input resistance r_n,

in series with the input load resistance R_i at the same absolute temperature T. Thus, the total output noise root mean square voltage is:

$$V_n^2 = 4\,kT\,(R_i + r_n)\,.G^2\,.df \tag{22}$$

where G is the open-loop voltage gain (provided the output is not rectified, in the case of an AC amplifier), and df is the input band width (k is the Boltzman constant). One may replace df by its optical equivalent R_s (spatial linear frequency band width) in EQUATION 13. Introducing this value in EQUATION 20 shows that the quantity of information Q_n contained in the optical noise is the equivalent of narrowing the effective entrance aperture of the instrument; that is, the optical noise occupies a portion S_n of the total aperture S, whose perturbing influence measured by the factor $4\,S_n/\lambda^2$ depends upon the wave length of the radiations utilized. It is difficult to conceive the construction of an optical filter that would perform functions similar to those of the step-up input transformer of an electronic amplifier.* The performance is improved, however, when the factor S_n can be related to a noise of wave length longer than that of the signal, as evident from EQUATION 20. Methods of noise filtration by re-imaging at a different wave length, discussed by Buerger,[44] are direct applications of this relation. In the field of microscopy, the idea is to have the contribution to the background of a wave length λ_n different from that forming the diffraction image λ_i. In the second step, one inverts the role of the two radiation beams. If F_o is the focal length of the objective and F_e that of the eyepiece, the final magnification M is given by the ratio $M = (F_e/F_o)\cdot(\lambda_n/\lambda_i)$.

The theory does not specify the kind of information that can be transferred by a beam of radiations. Similarly, it does not set any limit upon the number of radiation pencils from independent sources that can be transmitted by an aperture of finite size. The foregoing discussion of the scanning problem indicates that at the resolution limit one can get the maximum accuracy on one kind of information (spacing) at the cost of sacrificing all the other kinds (such as width and depth of the details), the total remaining unchanged. Indeed, by properly screening an aperture, the ultimate resolution is far greater than that calculated by the Abbe formula, but the energy distribution in the region adjacent to a single detail becomes indefinite and the location of the detail is lost. One can thus be informed of the presence of very small details without being able to ascertain their exact location in the field of view. By means of multiple-interference techniques, for instance, one can determine the

*Referring to EQUATION 22, if a step-up transformer of turn ratio n is used, its effect is to change the input load resistance R_i to the much higher value $R_i\,.n^2$, thus reducing the relative influence of the factor r_n: the signal to noise ratio is increased.

depth of a scratch on a crystal surface to better than $\lambda/1000$, but it is impossible to measure simultaneously the width and the Cartesian coordinates in the field. Interferometric methods involving the use of three diffraction slits in coherent light[45] raise the practical limit to about $\lambda/10{,}000$. In general, it is possible to establish paired quantities that appear to be mutually exclusive or that can be treated like canonically related variables regardless of the observation scale. Other examples of such combinations were mentioned in the chapter on performance criteria. These observations lead to a principle of optical indeterminacy† formulated by Ingelstäm[46] which comes into play every time one reaches a resolution limit whose existence is due to the finite values of both the radiation wave lengths and the instrumental aperture.

A few more examples illustrate the power of the simplified theory to predict how to resolve weak signals out of a large random channel noise, using phase-shift methods resembling those employed in electronics. Such methods include, notably, the various phase-contrast techniques reviewed by Francon.[47] For a comprehensive survey it is necessary to adopt at least one definition of coherence. Self-luminous objects, or objects illuminated by a large source, although not strictly equivalent, are examples of incoherent systems. In both cases the intensities add up linearly on the wave front. Such systems act as two-dimensional low-pass spatial-frequency filters whose output does not include the phase information present at the input. On the contrary, in coherent systems all the energy originates from a single, punctual, monochromatic source. According to Zernicke definition,[14] coherence is a quantitative term δ varying from 0 to +1. The concept of coherence has been rather slowly accepted, probably because it is not evident per se to the naked eye, and almost all radiation detectors are sensitive only to sums of intensities and not to phase differences. The calculation of the entire image distribution is somewhat more complex with coherent radiation but, again, the complete development is not required for an understanding of the principal conclusions. An important property of such svstems is that the coherence factor remains constant throughout between the object points of an image or on an entire wave front surface. For instance, it is constant in an entire Young's fringe pattern. One demonstrates, further, that systems obeying this condition are necessarily linear with regard to both phase and radiation amplitude, but nonlinear with regard to intensities.

It is clear that the filtration problems are more easily solved when simple means of amplitude modulation can be found to separate the high- and low-frequency components of a signal from the low-frequency noise

†In its generalized form, Heisenberg's equation contains two such canonically related variables.[48] Many fail to note that the principle applies to any periodic phenomenon at any scale. Thus, biological systems studied by means of electromagnetic radiation probes fall under its aegis.

spectrum. For instance. one may use the simple demonstration device described above (page 162) to effect such a separation. The collimator should be adjusted to produce a parallel beam of light (which will be coherent on account of the small size of the entrance aperture. A second collimator is next used to reimage the aperture upon the plane of view, but a small dark spot or a small absorbing filter is placed at the conjugate focus of the first half of the apparatus (the alignment is quite critical and must be made by Foucault's method). The result is an attenuation of the low frequencies transmitted by the central portion of the conjugate plane and a remarkable sharpening of the edges of an object imaged by the second collimator. An interesting method of sharpening photographs is based upon the above experiment: that of reimaging with variable filtration developed by Croce.[49] In this method the negative is illuminated with coherent light in order to reduce the noise reaching the positive plate. In the above experiment the use of a small absorbing annulus instead of a disk would allow a preferential transmission of the low frequencies of the object, with resulting increased contrasts but hazy edges. The first circumstance is definitely superior from the point of view of the information content of the image.

Optical imaging of known periodic objects was considered in the foregoing. The general case is that of a random distribution of the periods with simultaneous variations of amplitude, as with a photographic negative. Here again, some degree of optimization can be achieved by using the negative itself, properly located in the restitution apparatus, as the frequency filter of band width exactly identical to that of the objects in the entire field. Contemporary technology is able to calculate coating functions that can improve or deviate the performance of instruments in any prescribed manner. Such coating functions amount, physically, to the designing of filmlike filters with distributed absorbances following a predetermined pattern. The introduction of such a filter in the region where it attenuates, for instance, the high-frequency response of the system, as seen above, is quite comparable to the introduction of a moment of inertia in the channel. The comparable circumstance in electronics would be, probably a method of inductive damping,[49] although the opportunity to investigate this point seriously has been lacking.

The reverse problem is that of reducing the disturbance of the principal diffraction pattern produced by the aperture edges of an instrument. If this is allowed to take place in coherent illumination, the noise produced is also coherent with the main signal, and is therefore nearly impossible to filter out, as observed by Venot.[50] The problem consists in reducing the effect of distant aberration (for angles larger than 10°). With such angular incidence, the edges of the entrance aperture introduce diffraction fringes some of which, of sufficient order (sometimes even the thirtieth is effec-

tive), disturb the central parts of the image. Methods of counteracting these distant effects are now referred to as apodization.

Such optical techniques or methods of amplitude contrast are derived directly from the original attempts to effect such filtration by Porter[51] in 1906 immediately following the generalization of the Abbe diffraction theory of microscope image formation. A discussion of the phase variation problems is too involved for consideration here. Still more complicated cases arise in practice, when radiation detectors, their amplifiers when used, and the sources of electric power employed, contribute to the total optical noise. The total average combined noise voltage $\bar{e}_n$ includes the the quantity given in EQUATION 22 plus the factor $\bar{e}_s = 2\ eIR^2\ df$, representing the amplified noise generated in all other parts of the system by a mechanism different from thermal emission. The corresponding power supply regulation problems were previously discussed[52] and some solutions of particular problems in the field of electrophoresis analysis were offered.[53] When extreme accuracy and stability are required, as for automation application,[40-42] a modified approach is necessary, and the combination of the optimized optical system plus the amplifier and recorder must be treated as a damped resonant system whose analysis is, again, beyond the scope of this discussion.

Conclusions

Optical observation of biological phenomena. A brief survey of the basic tenets of optics revealed the analogies between electronic and optical devices. The latter are diffraction-limited, noisy, linear-communication channels whose spatial-frequency response is inherently contained within limits defined by a generalized interaction principle. The development of the theory leads to an evaluation of the performance of optical systems by a rather simple method of frequency analysis. This method is easily accessible to experimentation and measurement. The theory predicts that selective frequency filtration methods should improve the performance in specific applications.

The sampling theorem was considered for its practical value. The form of this theorem used in optics is similar to that of the communication theory: $N_p = 2\,RT$, where T (sec.) replaces the length L. In both cases only a finite number of parameters is sufficient to fully characterize any any experimental curve made up of simple harmonic constituents, even though the curve may appear to be continuous. This observation, it was seen, greatly facilitates experimentation.

An interpretation of optical phenomena from the standpoint of the communication theory yields interesting results. It was pointed out that the net effect of total optical noise is to reduce the effective aperture S by a factor $4S_n/\lambda^2$. Since the Ss contain an expression of the cut-off frequency

R of the optical-filter system, it is possible to express the result of EQUATION 22) in terms of bits per millimeter or any other unit related to the area S. One thus arrives at a relation similar to the Shannon theorem for the maximum rate M_t of transmission of a continuous message in a noisy channel: $M_t = R \cdot \log_2$ (signal information + noise information per noise information), except that spatial frequencies are now involved. Further development of the theory similarly would conduct to the expression of M_t in terms of calories per line (page 176), thus directly linking the quantity of information received by the observer to Schroedinger's negentropy[16] extracted from the object under observation.

Furthermore, it was seen that there exist canonically related variable pairs that legitimate the expression of a generalized principle of optical indeterminacy. This new principle enters into play in the study of phenomena involving periodic disturbances every time some dimension limit is reached. The results then take on statistical significance. This instrumentally conditioned indeterminacy raises important questions relative to the interpretation of the images of biological objects.

Despite the tenuity of the radiation probes employed, an interaction is produced within the biological object, so that complete predictability must escape us.[54] Some comfort may be obtained if one considers that a total knowledge is perhaps not essential for an understanding of open systems in the steady state. Many of our illustrious predecessors, including Clausius, Guye, Kelvin,[55] von Helmholtz,[56] in particular, have advanced the suggestion that biological systems do not obey Carnot's principle and the laws of equilibrium. Today it is accepted that entropy cannot but increase in living organisms which, like species, evolve in the unique direction of increasing complexity, structurally, physiologically, and chemically. Indeed, Hill[57] was fully justified in exclaiming, ". . . but if there is no equilibrium in living matter, how dare we apply rules and formulae derived from the idea of equilibrium?" It is apparently fruitful to characterize living systems by their continuous state of becoming (von Bertalanffy,[58] Prigogine.[59] However, only a dynamic abstract description is congruent, and the materialistic hypothesis of identity based upon the stability of material content, the causa materialis (substance) of Aristotle, disappears. In its place one rediscovers the *apora* of Democritus;* only the *causa formalis* (the accidents – shape, structure, and organization) is permanent. Strangely, a similar position is revealed in contemporary physics, a field in which immortality is reserved for energy alone.

*The part of our knowledge of things referring to their material content, which appears to be the most convincing, is precisely that which induces the gravest doubt when it is incorporated into a tentative total synthesis. The remaining elements of knowledge are incorporated into the philosophical concept of shape or structure (*Gestalt*), which is most vividly explained by Schroedinger.[16]

This new philosophy predicts that a generalized principle of interaction applied to optical observations and measurements should prove more fruitful than the simple interpretation of the classic experimental method and its rigid Cartesian philosophy. If so, it may be that the greatest contribution of the Twentieth Century is the quantitative use of the object-subject entanglement in order to gain a deeper insight as to the probable nature of the object, and of the subject as well, in the case of certain surprising psychological experiments. Thus, when measurements are carried out to the limits of resolution at which statistical indeterminacy enters into play, causality yields to probability, and the results are true intellectual concepts whose scope is no longer limited within the confines of a strict causality. The usefulness of the new concepts is ascertained when they satisfy the last criterion of a scientific theory instead of a metaphysics; they remain statistically provable, to an approximation defined by a properly scaled indeterminacy principle, by the procedures of the causalist experimental methods.

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